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EFFECTS OF CHEMICALS ON GROWTH IN PARAMECIUM

By

A. H. ESTABROOK

Dissertation submitted to the Board of University Studies
of the Johns Hopkins University in conformity
with the requirements for the degree
of Doctor of Philosophy

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A. H. ESTABROOK

From the Laboratory of Experimental Zoology Johns Hopkins University

WITH ONE FIGURE

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INTRODUCTION

Most studies of growth have been made upon organisms composed of a multitude of cells, so that the growth observed is a complex resultant of the growth of the component cells. It is evident that in producing such a resultant, the method of growth of each single cell might be very different from that of the organism as a whole. The present paper examines the growth of a single cell, as affected by various agents, particularly chemicals.

There have been but few studies of the growth of single cells. Jennings[1] ('08) studied the normal growth of the infusorian Paramecium, showing that it follows a curve that is similar, in a general way, to that of the growth of a multicellular animal having determinate growth. Popoff ('09) investigated the relative growth of nucleus and cytoplasm in a number of infusoria. Balls ('08)) examined the growth of the Sore-shin fungus, particularly the effect of temperature upon it. In this organism the growth of the cell is indeterminate, as in many of the higher organisms.

The common infusorian Paramecium has served as object of the present study. The normal growth of Paramecium is thus summarized by Jennings:[2]

In the following investigation, I have endeavored to find out some of the factors determining growth in Paramecium, and to discover whether size may be permanently modified by external conditions. The effect of the following chemicals upon the growth of Paramecium has been studied: sodium chloride, the alkaloids nicotine and strychnine nitrate, and alcohol. The growth in pure water, containing no food or salts, has likewise been studied.

I wish here to acknowledge my indebtedness to Prof. H. S. Jennings, under whose direction the work was done, for valuable suggestions and criticism. I also wish to thank Prof. B. E. Livingston for his interest in the experiments on osmotic pressure.

[1] Loc. cit., page 441.

[2] Loc. cit., page 448.

MATERIAL AND METHODS

In all the experiments one pure line of Paramecium has been used, that designated by Jennings[3] as I_2. This was the largest race he isolated, measuring from 200 to 230microns, orabout one-fifth of a millimeter. All the specimens used are descended from a single pair of conjugants which he selected from a wild culture. Thus, by using animals all derived from a single pair, any possible differences due to diverse ancestry are excluded and only variation within a pure line has to be dealt with. The Paramecia were kept in stock in large flat dishes holding about 2 to 3 liters. The hay infusion in which they were kept was made fresh about once a week, by pouring off some old infusion and adding new hay and fresh water. The Paramecia were thus kept in good condition and afforded an abundance of material at all times.

Paramecia undergoing division were picked out from the stock culture with the capillary pipette, and put on a slide with a concave depression. At the moment when the two halves separated, one of the daughter cells was placed in a few drops of hay infusion in one concavity on a ground glass slide, the other daughter cell was placed in a similar concavity on the same slide, this latter depression containing the solution whose effect was to be studied. The time at which the halves were isolated was noted, and after a certain interval they were taken out, killed and measured separately. This gives a direct method of measuring growth, as the specimens, can be killed at any age desired. The control in hay infusion gives a normal curve of growth and acts as a check, while the other shows directly the effect of the solution upon the normal growth. The fact that the two halves come from the same mother cell gives an almost ideal condition of experimentation, since the two organisms studied are of the same age, their previous history is the same, and they are in the same physiological condition at the moment of separation.

The Paramecia were taken out by means of the capillary pipette, gathered into one or two drops of liquid and then killed by sud-

[3] Loc. cit., page 494.

denly adding a large amount of Worcester's solution (a saturated solution of $HgCl_2$ in 10 per cent formalin). When Paramecia are properly killed by this method no distortion takes place. They were then measured by projecting them with the Edinger projection apparatus, and measuring the image with a millimeter scale. Lenses of such combinations were used that three microns in the object were equal to one millimeter in the projected image. The measurements were then converted into microns by multiplying by three.

GROWTH IN DISTILLED WATER

Before studying the effects of chemicals as upon growth it is well to examine growth in a fluid containing as small an amount of dissolved chemicals as possible; that is, distilled water. Any growth taking place in distilled water will evidently not be due to food materials present in the medium nor is it likely to be due to any specific salts, since these are almost lacking. After the nature of growth in distilled water has been determined, the specific effects of substances dissolved in the water can be studied.

Daniel ('08) showed that if Paramecia are introduced directly from hay infusion (which has a high salt content) into pure distilled water, it is the sudden change that injures them. He also showed that they could be made to live in pure distilled water if they were introduced into it gradually.

The distilled water used in the experiments was made by Dr. G. F. White of the Johns Hopkins Chemical Laboratory. Ordinary laboratory distilled water was redistilled from sulphuric acid and potassium chromate, then distilled from barium hydrate and condensed in block tin. This water gives a conductivity of about 2×10^{-6}.

Effect of Pure Distilled Water

In the experiments, one half of a dividing specimen was placed in hay infusion, such as was known to be favorable to growth: the other was placed in pure distilled water. Before transferring

to the distilled water, the specimen was washed once in a considerable quantity of the same distilled water, in order to remove any trace of hay infusion remaining on it. At the end of five minutes specimens in the hay infusion had increased in length from 139.5 to 153.3 microns. Those in the distilled water had grown precisely the same amount. Now, however, the injurious action of the distilled water began to show by the fact that the specimens in it began to shorten and thicken, while those in the hay infusion continued to increase in length and decrease in thickness. At the end of fifteen minutes the control specimens measured 159.6 × 42.7 microns, those in the distilled water 149.7×53.1 microns. The latter are thus actually shorter than they were ten minutes earlier. At the end of thirty minutes they were all dead, while those in the control were strong and active. The comparative measurements are given in Table I.

TABLE I

Comparative measurement in microns of Paramecium in pure distilled water and in hay infusion

AGE	CONTROL HAY INFUSION			PURE DISTILLED WATER		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.5	42.6	9			
5	153.3	44.0	12	153.3	47.0	12
15	159.6	42.7	13	149.7	53.1	13
30	Normal		15	All dead	Swollen	15

It is notable that a certain amount of growth did occur in the distilled water in the first five minutes, in spite of the fact that the latter is quickly injurious and soon fatal. It seems clear that the injurious result was here due, as in Daniel's experiments, to the sudden change from the fluid with the high salt content to one with a low salt content.

Effect of Adding NaCl to the Distilled Water

The effect of adding a small amount of some salt to the distilled water was next studied. For this purpose sodium chloride was used. When enough sodium chloride was added to the distilled water to give a $\frac{N}{100}$ solution, the results are those given in Table II.[4]

TABLE II

Comparative measurements in microns of Paramecia on $\frac{N}{100}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{100}$ NaCl		
	Length	Width	No. of Specimens	Length	width	No. of Specimens
Min.						
0	139.5	42.6	9			
30	167.2	38.7	8	164.3	39.3	9
90	177.3	37.9	14	169.5	37.5	14
Hours						
5	185.7	41.6	15	165.0	37.2	15
24	174.9	54.9	(10to)17	184.8(5) 5 alive	48.6(5) 5 dead	10

From this table it is evident that the addition of the small amount of the NaCl to the distilled water has a very good effect on the vitality and growth of the animals. Most of them are alive at the end of twenty-four hours. The growth is only slightly retarded in the salt solution as compared with that in the hay infusion. At the end of twenty-four hours, however, half the animals in the salt solution were dead or dying. The living specimens in the salt measured 18.48×48.6 microns, and were thus larger than the controls, which were 174.9×54.9 microns. But it must be noted that seven of the ten specimens that were in the

[4] In this and the following tables, whenever division has taken place, the figure enclosed in parentheses is the number of specimens at the beginning of the experiment, the other number indicates the number of specimens at the end of the experiment. In this case there were 10 specimens at the beginning of the experiment. These increased by division to 17 specimens at the end, all of which were measured.

control at the beginning had divided, giving seventeen specimens, and the difference in sizes is due to the fact that some of these seventeen specimens are young and not fully grown. I should say however, that the five in the salt were not quite so large as normal. Evidently growth that is almost normal in amount and rate can take place in a fluid that will not sustain life for any great length of time.

The effects in this experiment are explained by the one next performed. The animals were placed in pure distilled water plus a sufficient amount of sodium chloride to make a $\frac{N}{70}$ solution. The results are shown in Table III.

TABLE III

Comparative measurements in microns of Paramecia in $\frac{N}{70}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{70}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. .of Specimens
Min.						
0	148.5	50.7	8			
90	183.7	45.0	12	188.7	47.1	11
Hours						
5	200.1	58.2	14	177.3	45.9	12
24	199.2	63.9	(21 to) 27	211.5	60.9	21

As the table shows, growth in $\frac{N}{70}$ NaCl took place almost as well as in the hay infusion. At 90 minutes, those in the salt were as large as those in the control. At 5 hours, they had fallen somewhat behind, but at 24 hours they were of about the usual adult size. The lack of food had prevented them from dividing, while 6 of the 21 control specimens had divided, giving 27 altogether. The larger average size of the specimens in the salt solution was doubtless due to this division in the hay infusion; some of the specimens in the latter were young and not full grown.

Thus a $\frac{N}{70}$ solution of NaCl in distilled water has the necessary amount of salts for Paramecium to live in it with normal growth. The amount of salts in the $\frac{N}{100}$ solution was evidently not sufficient to keep the animals alive for a long time.

A $\frac{N}{50}$ NaCl solution in pure distilled water also gave interesting results, as shown in Table IV.

TABLE IV

Comparative measurements in microns of Paramecia in $\frac{N}{50}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{50}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.5	42.6	9			
30	171.6	37.8	10	164.1	40.5	10
90	182.0	37.8	15	166.6	37.5	15
Hours						
5	191.4	41.1	15	161.7	36.6	14
24	183.0	58.8	(10 to) 13	185.1	46.8	10

At the end of 30 minutes, the Paramecia in the $\frac{N}{50}$ NaCl solution were shorter than the controls. Growth was still retarded at 90 minutes and at 5 hours. At the end of 24 hours the specimens in the salt were long but not so thick as the controls. The controls, however, had increased from 10 to 13 specimens, so that those in the salt were only slightly retarded in growth. Those in the salt showed evidences of injury.

TABLE V

Comparative measurements in microns of Paramecia in $\frac{N}{20}$ NaCl dissolved in pure distilled water, and in hay infusion

AGE	CONTROL			$\frac{N}{20}$ NaCl.		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	146.4	46.2	10			
5	151.0	47.7	9	153.9	44.4	11
15	159.9	47.4	13	157.2	44.7	10
30	168.3	43.5	10	164.4	47.4	9
60	189.0	42.0	13	166.5 (2)	43.5 (2)	13
				11 dead	2 alive	

A $\frac{N}{20}$ NaCl solution in pure distilled water had no effect on growing Paramecia at 5 or 15 minutes of age (see Table V). At 30 minutes, those in the salt were slightly smaller than the controls; while at 60 minutes, of 13 specimens in the salt, 11 were dead and 2 were smaller than the controls.

To sum up the results with distilled water, we find that pure distilled water killed Paramecia that have been living in hay infusion by the great change from the high to the low salt content. When the Paramecia are put into distilled water with enough NaCl present to make it a $\frac{N}{100}$ solution, the injurious effect is not so marked and becomes very noticeable only at 5 to 24 hours. A $\frac{N}{70}$ NaCl solution in distilled water seems to have the necessary salt content to keep the Paramecia in normal condition. The $\frac{N}{50}$ and $\frac{N}{20}$ NaCl solutions in distilled water have a much higher salt content than is beneficial, and so cause a certain amount of inhibition of growth. More light upon this matter is given by the further work upon NaCl which is to be reported next.

GROWTH IN SODIUM CHLORIDE

Perhaps the chief purpose of the present paper was to study the effects on cellular growth of certain well-known poisons, particularly nicotine, alcohol and strychnine. In order that there should be no danger of mistaking for specific effects of these poisons symptoms that are likewise produced by other and unrelated chemicals, I first made a careful study of the effects of ordinary sodium chloride when added to the infusion in which the animals live. This substance, though not commonly accounted a poison, has of course most deleterious effects when present in too great amounts. These effects, particularly on growth, we shall now examine.

A $\frac{N}{10}$ NaCl solution was made by dissolving 0.146 grams of soldium choride in 25 cc. of hay infusion. This being the most concentrated solution of the salt that was used, any strength desired was made by dilution of this with hay infusion. The same hay infusion without the sodium chloride was used for the control experiments.

Preliminary Experiments on the Effect of NaCl on the Form and Dimensions of Adults

Adult Paramecia put into a NaCl solution died after some time. They first became thin, then shorter and much thinner, and slowly died. The appearance of Paramecia as they are dying is very characteristic; they become much swollen, sometimes twice as thick as normal, sometimes longer, or they may be shorter; the protoplasm contains many vacuoles; the animals swim about slowly. The exact moment of death is difficult to determine in this condition, as vitality seems to remain a long while, and even after they have become motionless, they may sometimes be made to move again by stimulating them with a capillary rod.

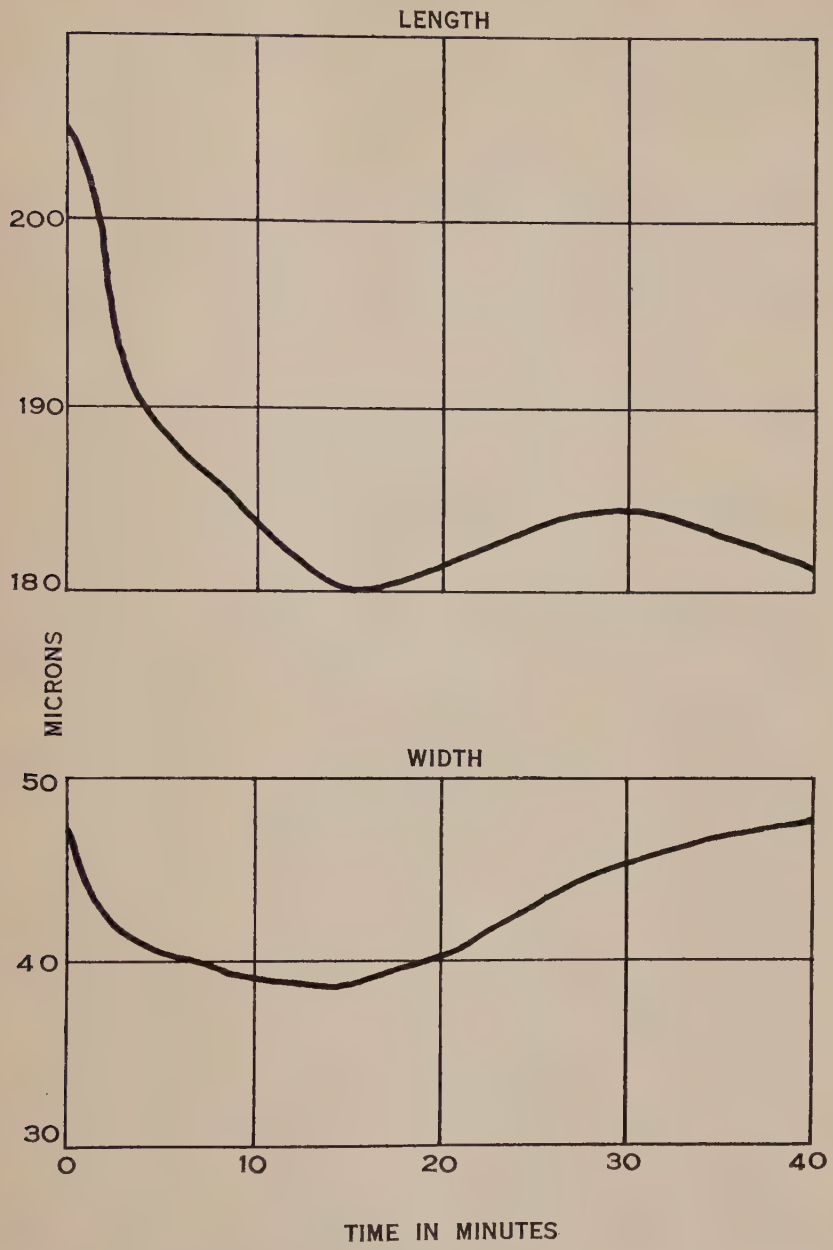
To determine the effect on dimensions, a large number of specimens were put into a $\frac{N}{10}$ NaCl solution (see table VI).

TABLE VI

Dimensions of adult Paramecia in $\frac{N}{10}$ NaCl.

TIME	LENGTH	BREADTH	NO. OF SPECIMENS MEASURED
Min.			
Normal at beginning	204.5	47.0	50
3	191.2	41.2	50
5	188.6	40.8	50
8	186.0	39.7	50
10	183.8	39.3	50
15	180.1	38.7	50
25	183.8	43.4	50
40	181.2	45.7	50

At intervals of 3 minutes, about 50 were taken out, killed and measured. Most of the animals died about 10 to 20 minutes after being put into the salt, but some, more resistant than the others, remained alive 60 minutes. The curve (Fig. 1), plotted from these figures, is thus based on different numbers of individuals


LENGTH
200
190
180
MICRONS
WIDTH
50
40
30
0
10
20
30
40
TIME IN MINUTES

in different parts; it is nevertheless instructive. The changes in the form and size of Paramecium shown by the curve, are the same as seen under the microscope. The first effect is an immediate decrease in the width, and at the same time, a shortening of the organism. Further decrease in length takes place, and then, as death approaches, the Paramecia become thicker and slightly longer. I have designated this form of curve as the death curve, as later work shows that its appearance is always followed by death.

Adult Paramecia put into a $\frac{N}{10}$ NaCl solution died in one-half to 3 hours, none living in this strength longer than 3 hours. The same general effects were observed as in the $\frac{N}{10}$ NaCl solution, except that, extending over a longer period, they were not so marked.

Adult Paramecia put into a $\frac{N}{20}$ NaCl solution became thinner for about 20 minutes, then gradually regained their normal size and continued to live normally in that solution.

In order to see if $\frac{N}{20}$ NaCl and the more dilute solutions of NaCl had any effect upon size in adult Paramecia which were kept in them for longer periods of time, the following experiment was tried: One hundred Paramecia taken from the stock culture were put into each of the following solutions, $\frac{N}{20}$, $\frac{N}{30}$, $\frac{N}{40}$ and $\frac{N}{50}$ NaCl, and a control composed of hay infusion (the same as that used for the solvent in the NaCl solutions). The Paramecia remained in the solutions 48 hours, were then taken out, counted, killed, and measured. This was done several times but as the results were in every case the same, only one series is reported here (see table VII).

TABLE VII

Dimensions of Paramecia that have remained 48 hours in various strengths of sodium chloride, compared with controls. Measurements are in microns and each is the average taken from 50 specimens. 100 specimens in each watch glass at beginning.

	NO. OF SPECIMENS AFTER 48 HOURS	LENGTH	WIDTH
Control	194	190.2	48.5
$\frac{N}{20}$ NaCl	220	173.5	49.0
$\frac{N}{30}$ NaCl	180	181.1	49.8
$\frac{N}{40}$ NaCl	210	188.4	49.1
$\frac{N}{50}$ NaCl	234	182.4	48.4

The table shows that these concentrations of NaCl have had no character stic effect either upon the rate of division or upon the size.

Only in the case of $\frac{N}{20}$ NaCl was there a slight indication of the modification of the rate of division, this concentration standing on the border line between the injurious and non-injurious solutions. In table VII, 100 Paramecia in $\frac{N}{20}$ NaCl had increased to 220, while an equal number in the control had increased to but 194. In another case, 50 specimens left in $\frac{N}{20}$ NaCl for 48 hours had increased to but 64, while in the corresponding control 109 had been produced.

Direct Measurement of Growth after Fission

Growth in solutions of NaCl was next measured directly by placing in the solutions to be examined specimens that had just divided, as described on page 491.

$\frac{N}{10}$ NaCl in hay infusion. The measurements in hay infusion to which NaCl had been added, and in the same infusion without the salt, are given in table VIII.

TABLE VIII

Comparative measurements in microns of growing Paramecia in $\frac{N}{10}$ NaCl dissolved in hay infusion, and in hay infusion.

AGE	CONTROL			$\frac{N}{10}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
5	152.9	50.4	18	153.2	37.2	16
10	162.5	52.2	22	161.7	37.2	11
20	166.9	51.7	14	162.1	39.1	15
40	186.9	49.6	19	141.5	42.0	11
65	182.4	50.1	18	142.6	41.4	11

For the first 10 minutes after separation, the halves in the salt solution increased in length at the same rate as the controls, then the length decreased rapidly for 40 minutes, when the Paramecia in the salt had become shorter than they were at the time of sepa-

ration. All those in the salt were dead at 60 minutes; many had died earlier; the latter, of course, were not measured. The width of those in the salt decreased very rapidly in the first 5 minutes, then remained the same up to 60 minutes, when the animals died.

$\frac{N}{20}$ *NaCl in hay infusion.* Table IX gives the measurements for growth when $\frac{N}{20}$ NaCl was added to hay infusion.

TABLE IX

Measurements of growth in $\frac{N}{20}$ NaCl *in hay infusion.*

AGE	CONTROL			$\frac{N}{20}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	144.3	52.2	7			
5	156.3	49.5	13	171.6	43.8	12
15	162.9	50.1	10	163.8	40.8	10
30	171.0	46.5	8	168.0	45.6	10
60	189.3	45.3	12	174.9	43.5	10
90	187.8	45.6	14	156.9	45.6	14
						6 alive
						8 dead
Hours						
6	All alive		50	44 alive		50
				6 dead		
25	198.6	58.5	(50 to) 56	181.8	52.1	
	All alive					
				23 alive		
				16 dead		11 Meas.
40	All alive		(56 to) 65	39 dead		50

For the first 20 minutes in this solution, the growth in length was normal. The width, however, showed a marked decrease, similar to that shown in the $\frac{N}{10}$ NaCl. At the end of 30 minutes, however, the Paramecia were normal in both length and width. At 60 and 90 minutes those specimens in the salt were shorter than the controls. After 90 minutes the Paramecia in the salt showed great variations in growth. Shortly after the age of 90 minutes specimens began to die, although some were alive at 30 hours. At the age of 40 hours none were alive in the salt, while

the controls had increased from 50 specimens to 65. At 25 hours, some of each were killed and measured. The controls measured 200.7 × 59.4 microns; those in the salt, 181.2 × 53.4 microns. This experiment shows that $\frac{N}{20}$ NaCl has a definite effect upon growth, inhibiting it after 60 minutes and finally causing death. It also shows that there is a great variation in the resistance of the animals to this strength of the chemical, some dying in 6 hours while other live 30 hours.

Growth in $\frac{N}{30}$ *NaCl.* In $\frac{N}{30}$ NaCl we have a strength of the chemical which may or may not affect growing Paramecia, depending on the conditions. In one of my experiments, there was apparently no effect of the salt upon the growing Paramecia at any time. Those in the salt grew to the same size as the controls (see table X).

TABLE X

Measurements of growth in $\frac{N}{30}$ NaCl.

AGE	CONTROL			$\frac{N}{30}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	144.3	52.2	8			
90	186.3	44.7	10	186.3	44.7	10
Hours						
24	192.3	66.	10	186.6	60.9	10

In another experiment, under what seemed exactly the same conditions as before, the $\frac{N}{30}$ NaCl had a slight inhibiting effect upon the growth. The measurements are given in table XI.

At the ages of 90 minutes, 5 hours, and 24 hours, those in the salt were shorter than the controls; sufficient numbers have been taken to give the figures some weight. I have worked out the probable errors for the averges, and find that, taking three times the probable error of each and substracting from the greater and adding to the less, in each case we still have significant differences, greater at 90 minutes and 5 hours than at 24 hours. Thus it seems from the analysis of the figures that there is in this case an

actual, though slight, inhibition of growth due to the $\frac{N}{30}$ NaCl. Thus $\frac{N}{30}$ is on the border line between a strength that will and one that will not affect growth in Paramecia.

$\frac{N}{40}$ *and* $\frac{N}{50}$ *NaCl in hay infusion.* Neither of these concentrations has any characteristic effect on the growth. See tables XII and XIII.

Rôle of Osmotic Pressure in Effects of Sodium Chloride

We may now ask whether these effects of NaCl are due to the osmotic pressure of the sodium chloride in solution acting on the Paramecia, or to the direct chemical effects of the substance, or to both.

By means of freezing point determinations,[5] we find that the osmotic pressure of ordinary hay infusion is .44 atmospheres. In table XIV is given the osmotic pressure of the different solutions of sodium chloride which we have used.

By examination of the table we find that an increase in the osmotic pressure from .44 atmospheres (that of hay infusion), to 1.841 atmoshperes (that of $\frac{N}{30}$ NaCl in hay infusion), has only a slight effect on the growth. Growth that was almost normal took place in $\frac{N}{30}$ NaCl in hay infusion. But when we increase to an osmotic pressure of 2.528 atmospheres, that of $\frac{N}{20}$ NaCl in hay infusion, there is a marked effect on the growth, which is even more marked at the pressure of 4.546 (that of $\frac{N}{10}$ NaCl.) This would seem to indicate then that an increase in osmotic pressure had an injurious effect on growing Paramecia. To give

[5] The osmotic pressure of the hay infusion has been found by the lowering of the freezing point method. When the lowering of the freezing point has been determined by the Beckman apparatus, the osmotic pressure is computed from the following forumula:

$$\text{O. P.} = \Delta \times \frac{22.3}{1.86^\circ}$$

Where Δ is the lowering of the freezing point, 22.3 is the osmotic pressure of a weight normal solution, and 1.86° is the lowering of the freezing point caused by a weight normal solution. The freezing point of a sample of hay infusion such as was known to be favorable to the growth of Paramecium was found to be .036 below that of pure distilled water, and by applying the above forumla the osmotic pressure of the hay infusion was .44 atmospheres.

TABLE XI

Measurements of growth in $\frac{N}{80}$ NaCl.

AGE	CONTROL			$\frac{N}{80}$ NaCl		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.0	50	10			
90	198.3 ± .987	42.1	20	176.55±1.230	45.1	20
Hours						
5	119.59±1.476	41.6	34	190.80± .897	43.8	35
24	206.17±1.758	55.5	18	192.34±2.136	57.2	18

TABLE XII

Measurements in growth of $\frac{N}{40}$ NaCl

AGE	CONTROL			SALT		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.8	53.4	9			
30	174.0	49.5	9	174.0	46.8	10
90	201.9	45.9	17	201.0	45.3	17
Hours						
5	200.7	44.4	20	196.2	46.5	21
24	219.4	63.6	14	224.8	64.8	15

TABLE XIII

Measurements of growth in 50 NaCl

AGE	CONTROL			SALT		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	131.1	49.8	7			
5	145.2	47.7	7	145.2	48.9	7
15	159.3	47.4	9	160.5	46.8	10
30	168.9	45.0	11	171.3	43.2	11
90	194.4	45.6	19	189.3	46.8	19
Hours						
5	194.7	42.3	20	193.8	43.8	20
24	198.4	60.0	22	202.9	58.2	20

further light upon the part played by osmotic pressure, a .079 N solution of cane sugar was made by dissolving 2.681 grams of sugar, which had been rendered free from all impurities, especially sodium chloride, in 100 grams of pure water. This solution has an osmotic pressure of 2.087 atmospheres and is isotonic with a $\frac{N}{20}$ solution of NaCl in distilled water.[6] Halves of Paramecia just divided were used, one-half put in the sugar, the other half in the NaCl solution, both being carefully washed free of any hay infusion. Now if the injurious action upon Paramecia which we have observed is due to osmotic pressure alone, we would expect the two solutions to have the same effect upon the animals in this experiment. The results are shown in Table XV.

Up to the age of 90 minutes, the specimens in the sugar did not grow as large as normal, but did grow faster than those in the salt. At 5 hours, those in the sugar were slightly larger than at 90 minutes, but still smaller than normal, while those in the NaCl were dead. At 24 hours those in the sugar were not as large as at 5 hours, but it must be noted that there was no food in the sugar solution. The animals in the sugar were alive, but small, however, at 48 hours. This experiment then shows that osmotic pressure has an effect upon growing Paramecia, but that the injury caused by the sodium chloride is not due entirely to the osmotic pressure of the solution, but is also due to some other factor. The effects caused by the action of the sodium chloride then, are due to the osmotic pressure exerted by the sodium chloride plus the direct action of the salt upon the protoplasm of the Paramecium. So far as is known the action of the sugar is due entirely to its osmotic pressure.

To see the effect of even higher osmotic pressures upon Paramecium, growth in a $\frac{N}{10}$ NaCl in hay infusion was compared with the growth in an isotonic solution of sugar, *i.e.*, a .16 N solution in hay infusion. Growing Paramecia were used, and it was found that the Paramecia died in both of these solutions at about the same time, 10 to 15 minutes. The inability of the Paramecia

[6] The measurements for the osmotic pressure of sugar solutions are taken from some unpublished work of Prof. H. N. Morse of the Johns Hopkins Chemical Laboratory.

TABLE XIV.

The osmotic pressures in atmospheres of different strengths of NaCl at 20° C. dissolved in distilled water and in hay infusion. The osmotic pressure of hay infusion is assumed to be .441 atmospheres.

STRENGTH OF NaCl	O. P. OF NaCl DISSOLVED IN DISTILLED WATER	O. P. OF NaCl DISSOLVED IN HAY INFUSION
$\frac{N}{10}$	4.105	4.546
$\frac{N}{20}$	2.087	2.528
$\frac{N}{80}$	1.40	1.841
$\frac{N}{50}$	.870	.....
$\frac{N}{70}$	.658	....
$\frac{N}{100}$	.432	.....

TABLE XV.

Comparative measurements of in microns Paramecia in $\frac{N}{20}$ NaCl in distilled water, and in .079 N sugar in distilled water.

AGE	GROWTH IN $\frac{N}{10}$ NaCl IN DISTILLED WATER			GROWTH IN .079 N SUGAR IN DISTILLED WATER		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	140.7	50.4	10	140.7	50.4	10
10	142.5	42.0	10	158.4	45.0	9
30	164.4	42.7	12	165.0	43.2	12
90	163.5	45.3	13	173.1	47.0	15
Hours						
5	All dead	....	10	175.5	50.1	10
24	All dead	....	..	154.2	48.6	11
48		....	..	Alive	....	10

TABLE XVI.

Table showing different effects of $\frac{N}{20}$ NaCl upon adult and young Paramecia.

AFTER HOURS	ADULT, 32 SPECIMENS		YOUNG, 32 SPECIMENS	
6	*Alive*	*Dead*	*Alive*	*Dead*
12	32	..	19	13
40	32	..	4	28
	28	4	3	29

[7] The data for osmotic pressure given here have been compiled from the formulæ given on pages 41–44 in "The Rôle of Diffusion and Osmotic Pressure in Plants," by B. E. Livingston, Chicago, 1903.

to grow in these solutions, which have a large amount of the chemical present in them, is probably due as much to the extraction of water from the cells as to the direct action of the chemical upon the life activities.

Comparative Resistance of Adult and Young Paramecia to $\frac{N}{20}$ *NaCl*

Early in the work it was noticed that adult Paramecia put into a $\frac{N}{20}$ NaCl solution continued to live, the solution having apparently no effect upon them, while young Paramecia put into the solution just after division died, before the end of 30 hours (as set forth above). A careful test of the varying resistance in the two cases was made as follows: Into each of the two depressions of a single slide was put a few drops of $\frac{N}{20}$ NaCl solution. Into one was put an adult Paramecium, into the other a young one from a fission just completed; both the adult and the young coming from the same stock culture, so that the past history would be nearly the same. Thirty-two pairs such were arranged and their further fate observed. The results are shown in Table XVI.

This experiment and other data given above (page 500), shows that adult Paramecia may live normally and grow in a solution that sooner or later kills Paramecia if they are put into the solution just as the two halves separate at division.

Are Paramecia in Stages Preparatory to Division Like Adult or Like the Young in their Resistance to the Salt?

To test this the following experiment was tried: Paramecia which were preparing for division were selected. They were then grouped in pairs, those being put together which were in the same stage of preparation for fission. (This is very easy to do with much accuracy, since one accustomed to working with Paramecia can tell within a few minutes when a dividing Para-

mecium will separate). One of a pair was put into hay infusion, the other one of the pair into a $\frac{N}{20}$ NaCl solution in hay infusion. It was found that all the pairs separated within 15 minutes of each other, so that there is little room for error. They were allowed to grow for 24 hours, and then killed and measured separately. Of 20 sets of pairs the results are as follows:

Control; hay infusion; 17 dividing Paramecia gave 34 specimens measuring at the age of 24 hours: $213.264 \pm 1.566 \times 71.19$.

3 dividing Paramecia gave 6 normal specimens not measured.

$\frac{N}{20}$ NaCl in hay infusion; 17 dividing Paramecia, mates of those in control experiment, gave 34 specimens measuring at the age of 24 hours: $205.059 \pm 2.466 \times 71.64$.

3 dividing Paramecia gave 6, all died.

Taking into consideration the probable errors of the measurements, there is evidently no significant difference in size of the two sets. However, three of the dividing Paramecia in the sodium chloride, after separating to form 6 specimens, died, while the corresponding controls remained normal. Why six out of forty in the NaCl should die, while the rest throve as well as the controls, is difficult to explain. Possibly there is a critical early period in which injury may occur; if the animals survive this, they flourish. The experiment taken as a whole shows, however, that the chemical is not so injurious to the young if the parents have been subjected to it a short time before fission.

Acclimatization to NaCl

We have seen above that certain concentrations of NaCl decrease growth. It was therefore determined to cultivate Paramecia in one of these solutions, to see if a smaller race could be obtained from L_2, or if possible, this race might changeinto one of the other races described by Jennings.[8] It was very easy to acclimatize Paramecium to a $\frac{N}{10}$ NaCl solution. If introduced directly into such a solution, Paramecia, as we have seen, die in a few minutes. The method of procedure for producing acclimatization was as follows. Adult specimens were put into a

[8] Loc. cit., page 494.

$\frac{N}{20}$ NaCl solution for 24 hours, then for a like period into $\frac{N}{15}$, then into $\frac{N}{10}$ where they will now live and multiply.

Fifty Paramecia were put into hay infusion and 50 into $\frac{N}{10}$ NaCl in the same hay infusion. At the end of 24 hours, 50 of the control were put into fresh hay infusion, and 50 of those in the $\frac{N}{20}$ NaCl were put into $\frac{N}{10}$ NaCl. At the end of the 24 hours, there were, as the result of some division, 69 in the control culture, 64 in the salt. Forty-four of the control were put into fresh hay infusion, and the 64 in the salt were transferred to the $\frac{N}{10}$ NaCl. At the end of 48 hours, the control numbered 54; those in $\frac{N}{10}$ NaCl, 75. Fifty of each were killed and measured. The average measurements of the 50 controls were 188.7 × 64.8 microns, of the 50 in $\frac{N}{10}$ NaCl, 170.7 × 70.02 microns. The Paramecia in the salt, as well as those in the control, were large and active. There was plenty of bacterial food present in both, the only evident difference being the smaller size of those in the NaCl. After four days, the specimens in the salt solution stopped dividing, while some had divided in an abnormal way. In many the division was incomplete, so that two specimens remained connected. Nothing of the sort was seen in the controls, the latter continued to divide normally. After the Paramecia had been in the $\frac{N}{10}$ NaCl 6 days, many began to die. An attempt was made to save these by putting them into fresh hay infusion containing $\frac{N}{10}$ NaCl, but when a few were introduced into this from the old culture, they all died in one to two hours, while those remaining in the old $\frac{N}{10}$ NaCl solution continued to live for nearly 20 hours more. The controls remained normal and alive.

Thus it is easy to acclimatize Paramecia to $\frac{N}{10}$ NaCl for a short period, but it is more difficult to get them into perfect relation to their changed environment. The animals in the salt at the latter end of the experiment were evidently in a very unstable equilibrium with their environment. Possibly the effect was caused by the accumulation of the sodium chloride in the cells.

Summarizing the work with sodium chloride, we find that the addition to the culture medium of sufficient NaCl to make a $\frac{N}{10}$ or $\frac{N}{20}$ solution permits the young specimens to grow for a few minutes after fission, then inhibits growth, finally causing death.

$\frac{N}{30}$ NaCl is on the border line; it sometimes decreases growth, sometimes not, dependinig on the conditions. More dilute solutions do not effect growth. Young specimens are injured by $\frac{N}{20}$ NaCl, while old ones are not. If the parents are subjected to this concentration for a short while before fission, the injury to the young is less. Paramecia can be temporarily acclimatized to a solution that would at first kill them, but after some days in such a solution fission becomes abnormal and ceases and the animals die. The injurious effects of sodium chloride are due partly to increase of osmotic pressure, partly to specific chemical action.

EFFECTS OF NICOTINE ON GROWTH

Having studied the effects of the comparatively innocuous substance, NaCl, I now undertook to examine the effects of nicotine ($C_{10}H_{14}N_2$). This alkaloid is so poisonous that only minute quantities are required to produce an effect; so minute that the osmotic pressure produced by them is clearly of no importance in the results. The effect of nicotine on single cells is of interest in view of its effect on man (see Lee, Langley, and others).

Effect of Nicotine upon Adult Paramecia

One hundred adult specimens of Paramecia taken at random from the stock culture were put into watch glasses containing known amounts of nicotine dissolved in hay infusion. These were allowed to remain for 50 hours. The results are given in table XVII.

From this table it is clear that strengths of nicotine from 1–1,000 up to 1–20,000 kill adult Paramecia in different times varying from 2 hours to 50 hours. Those in the 1–40,0000 solution and the more dilute solutions continued to live. In order to determine whether there was any effect upon the size a number of specimens were taken from these solutions, killed, and measured (see table XVIII.)

TABLE XVII

Effect of different strengths of nicotine upon Paramecia. 100 specimens each in watch glass at beginning of experiment.

PARTS OF NICOTENE IN PARTS OF HAY FUSION.	STARTED FEB. 1, 1909, 12:30 P. M.				
	Feb. 1, 2 P. M.	Feb. 1, 5. P M.	Feb. 1, 9 A. M.	Feb. 3, 10 A. M.	Feb. 3, 3 P. M.
1–1,000	All dead	.....	.....	.....	.....
1–1,250	All dead but 2	All dead	.....	.....	.....
1–1,666	All dead but 60	Few alive	All dead	.....	.....
1–2,000	All dead but 30	All dead	.....	.....	.....
1–2,500	50 dead	More dying	All dead	.....	.....
1–5,000	50 dead	More dying	10 alive	All dead	.....
1–10,000	All alive	All alive	Many dying	6 alive	All dead
1–20,000	All alive	All alive	Some dying	5 alive	3 alive
1–40,000	All alive	All alive	All alive	All alive	All alive
1–80,000	All alive	All alive	All alive	All alive	All alive
1–100,000	All alive	All alive	All alive	All alive	All alive
Control hay infusion	All alive	All alive	All alive	All alive	All alive

TABLE XVIII

Dimensions of Paramecia that have remained 50 hours in various strengths of nicotine compared with controls; measurements are in micorns, and each is the average taken from 50 specimens.

	LENGTH	WIDTH
At beginning of experiment	219.4	46.3
Control at end	260.7	67.5
1–40,000	213.3	63.4
1–80,000	210.2	64.0
1–100,000	214.5	63.4

It is seen that the Paramecia in the nicotine were about the same size at the end of the experiment as they were at the beginning. The controls, however, had increased in size from 219.4 × 46.3 microns to 260.7 × 67.5 microns. This would seem to indicate that those in the nicotine did not grow at all durng the period of the experiment.

Effect of Nicotine on Growing Paramecia

Growth in 1-2,500 nicotine. Growth in nicotine was measured directly in the way previously described (page 491). One of the two products of fission was placed in pure hay infusion, the other in hay infusion containing nicotine. In a solution of 1 part of nicotine to 2,500 of hay infusion, the results are as shown in table XIX.

TABLE XIX

Measurements of growth in 1-2,500 nicotine.

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.0	53.0	10			
5	159.7	57.7	12	155.4	59.2	12
10	164.1	58.2	11	157.8	62.4	10
20	172.1	56.2	11	156.5	69.8	11
30	169.0	57.6	9	163.5	65.1	10
60	191.7	52.6	9	160.2	68.7	9
Hours						
2	All alive		11	4 alive	7 dead	11
5	All alive		11	11 dead		11

TABLE XX

Measurements of growth in 1-5,000 nicotine.

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	132.6	53.4	10			
5	151.2	55.0	9	149.7	52.2	10
15	156.3	53.1	10	152.1	54.3	10
30	167.7	51.6	11	162.3	54.7	9
60	185.4	50.1	11	168.3	53.1	9
Hours						
9	197.7	52.5	11	175.2	49.2	7
24	212.1	62.1	25	174.9	48.4	25
				8 dead	10 alive	7 measured.
48	All alive		18	18 dead		18

At the end of five minutes, though there was some growth, those in the nicotine were not quite so large as the controls. At 30 minutes, they were still growing, but continued to be smaller than the controls. At 60 minutes, they began to decrease in size. At the end of 2 hours, of 11 specimens in the nicotine, 4 were alive and 7 dead. It is to be noticed that some growth took place in a solution of nicotine which later killed the organism.

Growth in 1–5,000 nicotine. In one part of nicotine to 5,000 of hay infusion, growth took place in the same general way as in the 1–2,5000 solution (see table XX).

The Paramecia grew in the nicotine, but were not at any time so large as the controls. At the age of 9 hours, those in the nicotine measured 175.2 × 49.2 microns, while the controls were 197.7 ×52.5. At the age of 24 hours, those in the nicotine were beginning to die, and at 48 hours, all those in the nicotine were dead.

Growth in 1–10,000 nicotine. In a 1–10,000 solution of nicotine, the Paramecia grew at about the same rate as those in the 1–5,000 (see table XXI), remaining somewhat behind the controls.

The difference is not great, but the results indicate a slight inhibition of growth caused by this amount of nicotine. All those in the nicotine were dead at 45 hours.

Growth in 1–20,000 nicotine. The results with 1–20,000 nicotine were variable (see table XXII).

Up to 5 hours, the growth was not evidently affected. At later periods the specimens in the nicotine were sometimes smaller, sometimes larger, than the controls. At 24, and 48 hours, they were distinctly smaller. Thus the effect of such a quantity of nicotine shows only after a considerable period.

Growth in 1–30,000 nicotine. In 1–30,000 nicotine, growth took place as in the control (see table XXIII).

At the end of 24 hours, the measurements in the nicotine were 193.2 × 63.9 microns, while the controls measured 187.5 × 68.7 microns. The difference is without significance; it is partly due to the fact that one of the controls had divided, the two small specimens so produced reducing the average length.

TABLE XXI

Measurements of growth in 1–10,000 nicotine.

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	145.8	53.7	10			
5	153.6	51.3	9	152.1	55.8	10
15	166.8	52.5	10	163.2	54.3	9
30	175.2	51.3	10	171.6	48.9	9
60	192.9	53.7	12	183.3	53.7	10
90	189.9	50.1	9	185.1	51.0	12
Hours						
5	180.9	45.6	9	170.7	52.5	8
24	186.0	55.8	5	177.6	49.8	5
45	All alive		17	17 dead		17

TABLE XXII

Measurements of growth in 1-20,000 nicotine

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	141.9	48.0	9			
90	187.8	43.5	8	185.1	45.9	11
Hours						
5	180.0	45.6	10	188.4	48.6	10
24	187.2	56.4	10	173.7	54.9	9
48	195.2	62.4	(10to12	185.2	53.2	8

TABLE XXIII

Measurements of growth in 1–30,000 nicotine

AGE	CONTROL			NICOTINE		
	Length	Width	No. of Specimen	Length	Width	No. of Specimens
Min.						
0	139.8	49.5	10			
90	167.4	46.2	10	168.9	45.3	10
Hours						
5	175.8	44.4	5	180.6	44.4	5
24	187.5	68.7	(12 to)13	193.2	63.9	12

Different Resistonce of Young and Adults to Certain Concentrations

Interesting results were reached in the effect of nicotine upon Paramecia of different ages. Nine adult specimens were put into a 1–5,000 solution of nicotine. Nine specimens just divided were put into the same solution. At the end of 2 hours, 5 of the adult specimens were dead, while only 2 of the young were dead. At 20 hours, all 9 adults were dead, while all but 2 young were alive. At the end of 30 hours, all the young were dead.

Some adult Paramecia were put into 1–20,000 nicotine. These were all dead at the end of two days. Some specimens just divided, put into the same strength of nicotine, grew to normal size and lived for 5 days, but did not divide.

Thus in these grades of nicotine the young Paramecia are more resistant to the chemical than adult specimens. In NaCl, on the other hand, as has been shown, the adult specimens are more resistant than the young specimens.

Summing up the results with nicotine, we find that a certain amount of growth takes place in the stronger solutions of nicotine, though these same solutions later kill the organism. This is true for solutions up to and including 1–10,000. In 1–20,000 nicotine solution there was a very slight retardation of growth at late stages. In the 1–30,000 solution the nicotine had no effect on the growth.

EFFECTS OF STRYCHNINE ON GROWTH

Strychinne has long been known to be a powerful poison for protoplasm. The work of Schulze, Binz, and others has shown its action on Protozoa, but there are no studies as to its effect upon growth in smaller quantities than lethal doses. Calkins and Lieb have shown that, in small quantities, strychnine increases the rate of division in Paramecium, but they did not study its effect upon growth and size. It will be interesting to determine whether there is increased size at the time when the animals show the increased rate of division, described by the authors mentioned. The general effects of this powerful poison upon cellular growth are likewise of interest.

Effects of Strychnine upon Adult Paramecia

A $\frac{N}{1000}$ solution of strychnine nitrate was made by dissolvin .0387 grams of that salt in 1,000 cc. of distilled water. This solu tion then contained about one part of strychnine nitrate in 2,50 of water. As this was the strongest solution used, other strength desired were made by further dilution of this with hay infusion

The 1–2,500 solution of strychnine killed Paramecia in a very few minutes. A 1–12,500 solution caused them to swell up and become vacoulated almost immediately, although they remained alive and moving for several hours. With more dilute solutions the effect is not so immediate or marked. To determine the effect of the more dilute solutions, watch glass preparations (as with NaCl and nicotine) were made. Strengths of strychnine up to 1–37,500 killed a great many of the Paramecia. In the 1–50,000 there were as many at the end of 48 hours as at the beginning. The normal rate of division was maintained in the 1–125,000 and more dilute solutions. There was no effect at any time upon the size (see table XXIV).

TABLE XXIV

Table showing rate of division of Paramecia in different strengths of strychnine and in hay infusion. 100 specimens in each watch glass at beginning.

STRENGTH OF SOLUTIONS USED	NUMBER OF SPECIMENS AT END
Control I	179
Control II	217
Control III	200
1–12,500	3
1–25,000	23
1–37,500	83
1–50,000	99
1–75,000	182
1–125,000	162
1–150,000	198
1–200,000	203
1–250,000	193

Growth of Paramecium in Strychnine after Fission

Growth in strychnine was measured directly in the way previously described (page 491).

Growth in 1–12,500 strychnine. For the first five minutes after separation, those in the alkaloid grew to the same length as the controls. At 15 minutes, those in the strychnine decreased in length until they were as short as they were at the time of fission, and remained so for 60 minutes, when all were dead. The width of those in the alkaloid increased very rapidly in the first 30 minutes (see table XXV).

TABLE XXXV

Measurements of growth in 1–12,500 strychnine.

Age	Control			Strychnine		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	133.8	54.0	6			
5	154.8	54.0	8	153.0	59.2	8
15	165.0	53.4	5	134.4	69.0	5
30	178.5	52.2	7	134.7	82.2	9
60	All alive		10	All dead		10

TABLE XXVI

Measuremente of Growth in 1-50,000 strychnine

Age	Control			Strychnine		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	134.1	51.9	7			
5	143.4	50.7	10	145.8	51.0	10
15	151.1	49.2	11	150.9	49.8	11
30	160.5	47.1	12	155.2	51.7	12
60	182.7	43.5	11	153.7	53.2	12
90	189.0	42.9	11	164.1	48.1	11
Hours						
2	All alive		10	Swollen and dying		10

TABLE XXVII

Measurements of growth in 1-75,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No of Specimens	Length	Width	No. of Specimens
Min.						
0	137.4	49.2	10			
5	149.4	48.0	9	150.3	49.2	9
15	159.3	48.6	10	157.8	49.2	10
30	165.6	43.8	10	161.4	47.7	10
90	191.4	40.5	10	171.0	46.8	10
Hours						
5	190.8	40.8	12	163.8	49.8	11
24	All alive		13	3 barely alive; 10 dead		13

Growth in 1–50,000 strychnine. Normal growth took place in a 1–50,000 solution of strychnine for the first 15 minutes (see table XXVI).

Further growth then took place, but was not so rapid as in the control. At the end of 2 hours those in the strychnine were dying.

Growth in 1–75,000 solution of strychnine. For the first 15 minutes after separation, the halves in the strychnine increased at the same rate as the control (see table XXVII).

Further growth took place, but was not so rapid as in the control. At 24 hours, of 13 specimens in the strychnine 10 were dead and 3 dying.

Growth in 1–100,000 strychnine. Normal growth took place in 1–100,000 strychnine up to the age of 30 minutes, the specimens in the alkaloid growing to the same size as in the control (see table XXVIII).

Growth then continued, but not so great in amount as in the control. At 5 hours, those in the strychnine were much shorter than the controls, but at 24 hours they had almost reached normal size again. At the age of 48 hours, the Paramecia in the strychnine had not grown as much as the controls; 14 specimens

in the strychnine had not divided and measured 181.2 × 44.1 microns, while 14 specimens in the control had increased by division to 33 and these measured 197.4 × 51.6 microns. Thus we find that in the 1–100,000 strychnine normal growth takes place up to the age of 30 minutes, then further growth ensues, but is not so great in amount and the specimens do not become as large at 48 hours as in the control. Division is also stopped in this strength of strychnine.

Growth in 1–125,000 strychnine. This concentration has no effect on the growth (see table XXIX.)

TABLE XXVIII

Measurements of growth in 1-100,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No of Specimens	Length	Width	No. of Specimens
Min.						
0	142.1	52.8	8			
5	145.8	50.7	10	146.4	50.1	10
15	149.7	51.9	9	156.6	52.5	10
30	162.3	46.2	11	161.1	48.3	12
90	191.4	45.6	9	179.4	51.9	9
Hours						
5	183.5	47.1	11	153.3	47.4	12
24	199.3	70.5	20	196.3	49.5	22
48	197.4	51.6	(14 to) 33	181.2	44.1	14

TABLE XXIX

Measurements of growth in 1-125,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.2	49.8	8			
30	166.2	45.6	9	169.8	46.8	6
90	188.1	43.2	10	188.1	45.6	10
Hours						
5	191.5	46.4	13	194.8	48.0	15
24	206.3	52.6	17	206.1	59.7	18
48	220.5	55.5	(5 to) 10	212.7	54.6	(5 to) 8

Growth in 1–200,000 and 1–250,000 strychnine. It was noted above that certain investigators had found that minute quantities of strychnine increased the division rate in Paramecium; in order to determine whether increased size accompanied this increased rate of division, more dilute strengths were used. In both 1–200,000 and 1–250,000, growth was exactly the same as in the controls (see tables XXX and XXXI). There was no evidence at any time of either increased size or increased rate of division.

Comparative Resistance of Adult and Young Paramecia to Strychnine.

Twenty adult Paramecia and 20 young Paramecia from the same stock culture were put into 1–25,000 strychnine solution.

RESULT

	ADULT; 20 SPECIMENS		YOUNG; 20 SPECIMENS
After Hours	*Alive*	*Dead*	
2	18	2	Some swollen
5	18	2	All dead
12	9	9	All dead
24	2	18	All dead

The same experiment was again tried using 1–50,000 strychnine in place of the 1–25,000. The results follow.

	ADULT; 20 SPECIMENS	YOUNG; 20 SPECIMENS
After Hours		
6	All alive	Few swollen
6	All alive	Dying
11	All alive	All dead
24	13 dying, 7 dead	All dead

It is evident from these experiments that adult Paramecia have a much greater resistance than young specimens to the stronger solutions of strychnine.

Summarizing the effects of strychnine, we find that when specimens just after fission are introduced into hay infusion containing strychnine at concentrations of from 1–12,500 to 1–75,000 a

TABLE XXX

Measurements of growth in 1-200,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Hours						
0	145.0	50.0	10			
5	195.9	39.9	10	199.2	45.0	9
24	196.1	55.1	20	193.3	59.5	20
48	201.3	48.3	(29 to) 39	205.9	52.5	(29 to) 30

TABLE XXXI

Measurements of growth in 1-250,000 strychnine

AGE	CONTROL			STRYCHNINE		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Hours						
0	145.0	50.0	10			
24	193.8	50.1	10	191.1	55.5	10
48	196.4	44.3	(20 to) 32	203.8	48.7	(20 to) 27

TABLE XXXII

Measurements of growth in 5 per cent alcohol

AGE	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.3	44.4	10			
5	146.7	45.9	10	137.7	44.7	9
30	163.2	39.3	8	146.4	45.3	10
90	165.8	39.5	11	153.0	44.4	9
Hours						
3	176.1	39.0	11	156.9	53.7	10
5	All alive		15	Dead 15 sp.		15

certain amount of growth takes place in the first few minutes, then the animals die. In strychnine at 1–100,000, growth is almost normal in amount. No evidence appeared of increased size or increased rate of fission due to strychnine in any strength.

Adult Paramecia are more resistant than young specimens to the stronger solutions.

Effects of Alcohol on Growth

Introductory. So much has been written in the last few years, and such diverse results reached on the effect of alcohol upon higher animals that it will be interesting to study the effects of alcohol upon growth in single cells. Calkins and Lieb,[9] in 1902, showed that "alcohol had no effect upon Paramecium taken in too weak doses and too powerful an effect when taken in over strong doses," also "when a medium dose was given, *i.e.*, one part of alcohol in 2,500 of hay infusion, the effect is a continued stimulus which sustains the high rate of division even during periods of depression of the control series."

Woodruff,[10] in 1908, found that alcohol increased the rate of division at certain periods in the life cycle of Paramecium and decreased it at others. He also found that the increased rate of division was not lasting, but that doubling the amount of alcohol again caused a rapid cell division for a limited period.

It will be of value, then, to see what effect different amounts of alcohol will have upon growing Paramecia, and whether minute quantities will cause increased size or increased rate of division.

Effect of Alcohol on Growing Paramecia

Growth in 5 per cent solution of alcohol in hay infusion. Young Paramecia just after separation were used, in the way described on page 491. Some growth took place in a 5 per cent solution of alcohol in hay infusion, but the animals did not grow so fast at any time as the controls.[11] All those in the alcohol were dead at the end of 5 hours (see table XXXII).

Growth in 3 per cent alcohol. A certain amount of growth took place in 3 per cent alcohol, but the specimens in the alcohol did

[9] Loc. cit., page 364.

[10] Loc. cit., page 85.

[11] The slides containing the alcohol cultures of Paramecia were kept in a separate chamber from the the controls. In the moist chamber containing the alcohol cultures. the bottom was covered by a 5% solution of alcohol and in this way loss of alcohol by evaporation from the culture drops was prevented. The control cultures were of course kept in water vapor alone. In the other strengths of alcohol studied, similar precautions were taken.

TABLE XXXIII

Measurements of growth in 3 per cent alcohol

AGE	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	139.2	45.6	10			
30	162.0	39.3	10	153.9	39.6	10
90	187.0	38.2	18	173.1	39.3	16
Hours						
5	191.7	37.2	11	185.7	48.0	10
12	All alive		20	All dead		20

not at any time grow as fast in the alcohol as in the control (see table XXXIII). At 12 hours, all those in the alcohol were dead.

Growth in 2 per cent alcohol. At the age of 90 minutes, Paramecia had grown to normal length, but were not so thick as the controls. At 5 hours, they were slightly shorter in length and much thinner than the controls (see table XXXIV). At 24 hours, those in the alcohol measured 196.1 × 56.5 microns, and had not divided at all, while the controls had increased from 22 to 42 specimens. Thus in 2 per cent alcohol animals had grown to about normal size, but did not divide. The effect of the alcohol is shown mainly on the inhibition of division.

Growth in 1 per cent alcohol. At the end of 90 minutes, Paramecia growing in 1 per cent alcohol were slightly larger than the controls. At 5 hours and at 24 hours those in the alcohol had grown to the same size as the controls. Thus 1 per cent alcohol has no effect upon the growth (see table XXXV).

Growth in 1–500 alcohol. As was mentioned above, it has been found by certain investigators that minute quantities of alcohol cause increased rate of division. In order to see if increased size accompanied this greater division rate, the effects of more dilute solutions of alcohol were tried upon growing Paramecia.

Paramecia grew to exactly the same size in the 1–500 alcohol as in the control (see table XXXVI).

Growth in 1–2,500 alcohol. In a solution of 1 part alcohol in 2,500 of hay infusion, Paramecia grew to the age of 5 hours, at the

TABLE XXXIV.

Measurements of growth in 2 per cent alcohol.

AGE	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.0	45.0	10			
90	191.0	52.3	9	192.0	43.2	9
Hours						
5	190.2	45.7	9	186.3	36.6	9
24	22 specimens increased to 42		(22 to) 42	196.1	56.5	22

TABEL XXXV.

Measurements of growth in 1 per cent alcohol.

AGE	CONTROL.			ALCOHOL.		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.0	45.0	10			
90	189.0	42.6	9	197.7	43.3	9
Hours						
5	202.2	44.7	13	205.6	40.8	15
24	210.6	67.7	34	213.6	69.6	33

TABLE XXXVI.

Measurements of growth in 1-500 alcohol

AGE.	CONTROL			ALCOHOL		
	Length	Width	No. of Specimens	Length	Width	No. of Specimens
Min.						
0	135.0	45.0	10			
90	185.2	38.4	18	186.6	37.9	16
Hours						
5	190.5	38.4	21	187.4	41.1	21
24	204.7	64.8	29	202.0	63.1	30

same rate as in the control (see table XXXVII). At 24 hours, however, those in the alcohol were 6.2 microns longer than the controls. If the probable errors of the length are inspected, this

TABLE XXXVII

Measurements of growth on 1-2,500 alcohol.

AGE	CONTROL			ALCOHOL		
	Length	Width	NO. OF SPECIMENS	Length	Width	NO. OF SPECIMENS
Min						
0	133.8	40.5	10			
30	161.0	40.3	9	156.6	38.1	10
90	171.0	35.1	10	168.3	37.8	10
Hours						
5	179.7	40.5	10	178.6	38.7	9
24	199.7	63.6	21	205.9	65.5	20
	1.479			2.094		

difference becomes insignificant. So the result would indicate that the alcohol had not increased the growth above the normal amount.

Growth in hay infusion of Paramecia treated for 30 minutes after fission with 7½ per cent alcohol. To see if treatment with a strong solution of alcohol for a short period would have any lasting effect upon the animals when transferred to normal conditions, or whether the animals would be inhibited in growth for the actual time in which they were in the alcohol, the following experiment was tried: Paramecia just after fission were put into 7½ per cent alcohol and allowed to remain. At the age of 5 minutes, and 30 minutes, they were shorter than they were at the time of separation. They were also swollen and vacuolated and motionless. Water evidently had been extracted from the Paramecia by the strong alcohol (see table XXXVIII).

At the end of 30 minutes the Paramecia in the alcohol were taken out and put into hay infusion. Fifteen minutes after being taken out of the alcohol or at the age of 45 minutes, they had increased from 121.8 microns (the size at which they were transferred from the 7½ per cent alcohol to the hay infusion), to 147.9 microns, being then sightly larger than at the time of fission. No further growth took place in the Paramecia that had been treated for 30 minutes with the alcohol until shortly after 5 hours. At that time the Parmecia began to increase in size, and

TABLE XXXVIII

Measurements of growth of Paramecia treated with 7½ per cent alcohol for 30 minutes after separation and then placed in hay infusion

AGE	CONTROL Paramecia in Hay Infusion			EXPERIMENT			
	Length	Width	No. of Specimens		Length	Width	No. of Specimens
Min.							
0	142.2	44.4	10	in 7 ½ per cent alcohol			
5	144.6	45.0	9	in 7½ per cent alcohol	123.3	48.9	10
30	150.3	42.0	10	in 7½ per cent alcohol	121.8	54.0	8
45	175.5	41.0	9	30 minutes in alcohol, 15 minutes in hay infusion	147.9	41.7	10
60	187.9	42.7	8	30 minutes in alcohol, 30 minutes in hay infusion	144.7	46.6	9
Hrs.							
2	184.5	38.6	16	30 minutes in alcohol, 90 minutes in hay infusion	148.4	39.4	13
5	171.5	42.3	11	30 minutes in alcohol, 4½ hours in hay infusion	137.4	42.8	11
24	177.5	49.5	16	30 minutes in alcohol, 23½ hours in hay infusion.	160.6	51.7	13
48	187.3	53.7	(6 to)9	30 minutes in alcohol, 47½ hours in hay infusion	184.9	59.4	(6 to) 11

at 24 hours they measured 160.6 × 51.7 microns. They were at that time shorter than the controls, which had not been treated at the beginning with the alcohol. At the age of 48 hours, the control had increased from 6 to 9 specimens and measured 187.3 × 53.7 microns, while those that had been treated with the alcohol for 30 minutes after fission, and then placed in hay infusion, had increased from 6 to 11 specimens, and measured 184.9 × 59.4. At 48 hours the Paramecia had entirely recovered from the effects of the initial treatment with alcohol and had regained their normal size.

Thus we find that $7\frac{1}{2}$ per cent alcohol inhibits growth completely, and that the inhibitory effect lasts for some hours after the animals have been removed from it. But finally the inherent tendency to grow overcomes the effects of the alcohol, and the specimens regain their normal size and rate of growth.

Resistance of Adult and Young to Alcohol

There is no such marked difference in the resistance of a young and adult Paramecia to strong solutions of alcohol as we find with the other chemicals. In a 5 per cent solution young and adults die in about the same time (2 to 3 hours). In a 3 per cent solution the adults are slightly more resistant than the young, living in the solution about 12 hours, while the young die in about 5 hours.

The relation of alcohol to growth casts some light in the rôle of osmotic pressure in producing the effects of chemicals on growth. We find that nearly normal growth takes place in 2 per cent solution of alcohol; this has an osmotic pressure of 8.24 atmospheres, while Paramecia die quickly in a $\frac{N}{10}$ solution of NaCl, with an osmotic pressure of but 4.55 atmospheres. This indicates that changes in osmotic pressure have only a small part in the effects of these chemicals upon growth.

Thus the effects of alcohol on growth in Paramecium are in a general way similar to the effects of the other chemicals studied. The stronger solutions, 5 per cent and 3 per cent, inhibit growth somewhat, and the animals finally die. Normal growth takes place in 1 per cent alcohol. No evidence was found that minute quantities of alcohol increase growth. The adult Paramecia are slightly more resistant to alcohol than the young.

Effect of Food on Growing Paramecia

At almost every step in these experiments, the question arose as to what part the presence or absence of food plays in the growth of Paramecium. We have seen that during the first 90 minutes, growth took place very fast. To what is this rapid growth due? Is it due to ingested matter or to imbibition of water? Growing Paramecia were put into hay infusion containing India ink, and it was found that no particles of the India ink were ingested into the body of the animal until about 30 minutes after fission. Thus the marked increase in size which takes place in the first 30 minutes in a growing Paramecium cannot be due to ingested matter of any sort. The only other explanation for the increase in size is the imbibition of water.

To determine directly the effect of food upon growth in the early stages, the growth of Paramecium in tap water containing rather little bacterial food was compared with the growth in fresh hay infusion. Halves of divided specimens were put into hay infusion, the other halves of the same specimens into tap water which had been boiled and then aerated. At the end of one hour, 35 specimens in the hay infusion measured 173.1 × 40.9 microns, 35 in the tap water 171.4 × 40.6 microns. We find that the same amount of growth has taken place in both.

The growth in tap water as prepared above was then compared with that hay infusion in which there was dense bacterial growth. Specimens just after fission were used and kept in the different culture fluids one hour. At the end of that time, the Paramecia in the hay infusion with plenty of bacterial food present measured 169.8 × 42.0 microns (10 specimens), while those in the tap water with practically no bacterial food grew to about the same size, 171.3 × 42.3. From this experiment and the experiment before, growth to the age of 60 minutes would seem to be independent of the amount of food present, as the same amount of growth takes place in the tap water with no food present as in the hay infusion with the large amount of bacteria.

To see what effect food will have upon growing Paramecia for longer periods of time the following experiment was performed:

Some hay infusion was allowed to stand in a warm room until it became turbid from the growth of bacteria. Part of this was then filtered through a new Pasteur-Chamberlain bougie. The filtrate was thus rendered free of all bacteria and was also exactly the same, chemically and otherwise, as the hay infusion with the bacteria in it before filtering, except for the absence of the bacteria. Halves of dividing Paramecia were put into these two solutions, one with bacteria, the other without. At the end of 90 minutes, there was, as was to be expected, no difference in size. At the age of 5 hours, the Paramecia in the hay infusion with the bacterial food were shorter and thicker and measured 200.1 × 60.5 microns (14 specimens), while those growing in the media without bacteria were longer and thinner; 11 of these measured 210.9 × 45.0 microns. At the age of 24 hours the presence of food has had a marked effect. Twenty-eight specimens in the hay infusion with the bacteria had increased by division to 49 specimens, and these measured 193.5 × 62.2 microns, while the 28 specimens in the medium without food had not divided at all and measured 205.2 × 53.4 microns. It must be said here, however, that some growth of bacteria had taken place in the solution which was sterile in the beginning, as it is impossible to wash Paramecia absolutely free from adhering bacteria. Yet at 24 hours, there was evidently an abundance of bacteria in the one medium, and almost none in the other.

Thus in the early stages of growth up to the age of about 90 minutes the presence or absence of food material in the medium has no effect on the size of the growing animals. As we have seen earlier, the greatest amount of growth takes place in the first 90 minutes and our experiments indicate that this increase in size is due mainly to imbibition of water. From then on, the presence of food in the culture fluid has an effect on the size to which the Paramecia will attain. With plenty of food present the animals grow shorter and thicker, and divide sooner, than those kept in a medium with less food.

It is to be noted that in the experimental work in the general effects of the different chemicals studied, the question of bacterial food does not enter in the interpretation of the results. It was

found that in no case did the presence of any of the chemicals in the hay infusion stop the normal growth of the bacteria, the culture medium with the chemical in it becoming turbid with bacteria at about the same time as the control hay infusion. For this reason there will be about the same amount of food in one culture medium as the other, and the question of food affecting the general results will be negligible.

SUMMARY AND DISCUSSION

1. The investigations do not show that any of the substances studied have what could be called a specific or characteristic effect on growth. When very weak they have no effect whatever. In greater concentrations, all retard the later stages of growth, at the same time manifestly interfering with the other vital processes of the organism. When still stronger even the early stages of growth are impeded or prevented; in such cases the organism is quickly killed by the chemical. In no case is the growth affected without other injury to the organism. On the whole it appears clear that the effects on growth are secondary; they are consequences of the interference of the chemical with the other vital processes of the animal, and do not appear unless such interference exists. Essentially the same effects on growth appear whether the interference with other vital processes is due merely to the absence of any salts in the medium, to the presence of undue quantites of such a common substance as sodium chloride, or of minute quantites of such poisons as nicotine, alcohol or strychnine. None of the substances studied, whatever the amount present, has a tendency to increase the normal rate or amount of growth (although the presence of a small amount of sodium chloride permits this normal growth to occur, when it otherwise would do not so).

2. The early stages of growth show in certain respects a remarkable independence of the surrounding medium. In many cases, as we have seen, a certain amount of growth takes place under conditions which later destroy the organism. At fission the organism seems to have a certain potential of growth, due largely to

internal conditions; it has a strong tendency to grow in a perfectly definite way, at a definite rate, the rate giving a curve of a definite form. It grows for a time in this way in spite of the almost complete absence of the salts that are necessary for its continued existence, and in spite of the presence of actively injurious chemicals, which in a short time kill the organism. Whatever growth occurs tends to follow the normal growth curve. The substances within the organism, plus water, seem all that is necessary for this process, and it persists for a time in spite of positively injurious external conditions.

No evidence was found that a race of a given typical size can be transformed by any of the chemicals studied into a larger or smaller race. Their effects on growth seem due to interference with the vital processes, resulting in pathological conditions in other respects as well as in growth. Continued action of the chemicals that interfere with growth usually sooner or later cause death. The causes of the observed temporary changes in size in a given race under differing cultural conditions are probably to be sought for in variations in the nutritive and other conditions of the normal environment, particularly in conditions that affect the rate of fission.

3. On the precise nature of the deleterious action on growth in the case of the different chemicals the investigations gave little light. In the case of sodium chloride it appears possible, as we have seen, that a part of the injurious action is due to osmotic pressure, while a part is not. But in the case of nicotine and strychnine the minute quantites employed show that the osmotic pressure plays no part, yet these chemicals produce essentially the same effects on the growth as do undue amounts of sodium chloride. In the case of alcohol the growth occurs in solutions having a much higher osmotic pressure than the deleterious solutions of sodium chloride. Thus all the facts taken together seem to indicate that disturbance in osmotic relations plays little part in producing the effects on growth, even in the case of such substances as sodium chloride.

4. As to the processes in growth itself, it appears clear that the increase in size in early stages, up to 60 to 90 minutes (at

which time half the growth in length has occurred), is due almost solely to the imbibition of water. Up to this time growth occurs in much the same way whether there are food substances present in the water or not; as we have seen, it takes place when all the solid substances are removed from the fluid by filtering, or even in pure distilled water containing a little sodium chloride. After about ninety minutes the presence or absence of bacterial food in the medium has a noticeable effect on the growth. If plenty of bacterial food is present the animals are thicker, but do not grow so long as with little food; this is owing to the fact that where food is abundant, fission takes place more frequently. The animals may, however, reach the normal length in a medium containing almost no food; we have seen that this occurred in distilled water containing a little NaCl. But in such cases fission does not occur; this growth by mere imbibition of fluid can manifestly not continue for more than one generation.

5. In investigating the different resistance of young and adult Paramecia toward the stronger solutions of the chemicals, it was found that adult Paramecia were much more resistant than very young animals toward sodium chloride, strychnine, and alcohol, while the reverse was true with nicotine, the young being more resistant than the adult. It would be naturally expected from the work done on higher animals that the adult would be more resistant. The reversal of the effects found in nicotine are probably due to some specific effects of the nicotine which are not present in the other chemicals.

6. Acclimatization of Paramecium to strengths of sodium chloride, which would kill the animals if introduced directly, was found to be easy, but this acclimatization was not permanent and the animals finally died. In acclimatizing Paramecia to a $\frac{N}{10}$ NaCl solution the ratios of the different salts within the cell are probably changed to such a degree that it takes a long time for the cell to regain its equilibrium so as to continue its normal metabolic processes. Acclimatization of Paramecia to the other chemicals was not attempted.

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